

## Statistical and Other Issues

*This chapter provides an overview of some of the statistical issues involved in making DNA-based identifications of victims of a mass fatality incident. Because both mathematics and policy should be considered when determining statistical thresholds for making an identification, a single statistical approach may not be sufficient for every mass fatality disaster. Issues to consider when setting policy for a mass fatality response would include, for example, the condition of the remains, and the existence and reliability of samples. Appendix I contains an extensive reference list that may assist laboratory managers, policymakers, or public officials who desire a more indepth understanding of the use of statistics in making DNA-based identifications.*

When dealing with statistical issues—including the statistical threshold necessary to make a DNA-based identification of a victim's remains—it is important that the identification policy for a particular mass fatality response effort be consistent with the goals of the effort. Decisions about the number of and specific loci to type, the statistical thresholds, and the use of outside laboratories and consultants should be made quickly (see chapter 4, *Major Decisions*). For example, in the World Trade Center (WTC) identification effort, the Kinship and Data Analysis Panel (KADAP) endorsed the decision of the New York City Office of the Chief Medical Examiner (OCME) to use the standard Combined DNA Index System (CODIS) core 13 short tandem repeat (STR) loci, as well as the Amelogenin sex-typing locus used in forensic laboratories throughout the United States (see chapter 11 for more on “mini-STRs,” single nucleotide polymorphisms, and mitochondrial DNA sequencing, which also were used in the WTC identification effort).

It is important to note that the identification of WTC victims did not require the creation of any new statistical approach. In fact, the statistical approach recommended by the KADAP and used by the OCME was based on two well-established methods.

The first method, known as “direct matching,” assesses the probability or likelihood that a DNA profile from a victim's remains and a profile developed from a personal item known to belong to a missing individual would share—by chance—the same DNA profile. The direct matching method is similar to that used in forensic genetic testing, in which there is an estimate of the strength of a match between a DNA profile from biological evidence and a profile obtained from a known reference sample. Direct matching was used in approximately two-thirds of the WTC DNA identifications.

A second statistical method, called “indirect matching,” uses methods of formal genetic kinship analysis, in which a comparison is made of the DNA profile from a victim's remains and those of biological relatives in a known kindred (i.e., a “family tree” or pedigree). Also called “kinship analysis,” this approach is similar to that used for parentage assignment in paternity testing, nursery mixup resolution, immigration, and probate disputes. Kinship analysis was necessary in about one-third of the DNA-based identifications of WTC victims.

**In the field of human genetic research, genotypes of relatives have been used to reconstruct a partial or total genotype for the purpose of gene mapping. Experience gained in this area proved to be invaluable in helping to identify WTC victims.**

*Michael Conneally*

**The KADAP spent time considering and discussing the population makeup of the World Trade Center victims, including the population substructure that might influence the posterior probability of an identification. In the end, our consensus was to be conservative in all calculations to guard against false identifications.**

*George Carmody*

The theories and practices of statistical analyses in making DNA-based identifications are well developed and well documented. Before any DNA testing is performed, the “posterior probability”—that is, the level of confidence needed to make an identification—should be established. The posterior probability is based on the product (multiplication) of two components: a “prior probability” and a “likelihood ratio.”

**Prior probability** is the chance that any remains

sample belongs to a particular individual; typically, it is based only on the estimated number of reported missing persons (RM), which can change over the course of the identification process. In the WTC identification effort, for example, the number of RMs was originally much higher—as many as 5,000—than the final estimate of approximately 2,750, after multiple reports, multiple nicknames, and other victim data were reconciled.

**Likelihood ratio** is the strength of the DNA evidence favoring identification.

## Statistical Threshold

In the WTC identification effort, the statistical threshold (i.e., posterior probability) that was established before a DNA-based identification could be made was high: 99.9%. To attain this high level of confidence, the DNA laboratory targeted a likelihood ratio that, combined with the prior probability, met the statistical threshold. This likelihood ratio (**L**) was based on the following formula:

$$1 - (1-1/L)^N \leq p$$

In this formula, **N** = the estimated number of RMs (5,000 in the WTC); and **p** = the defined acceptable chance of error (1/1,000,000). Solving for the likelihood ratio:

$$L \geq 10,000,000,000$$

## Open vs. Closed Incidents

Mass fatality incidents have been described as either an “open” system or a “closed” system. Open systems are those in which the number and identity of victims is unknown. Closed systems are those in which the number and identity of victims is more certain, such as in a small car or airplane crash.

As noted in chapter 4, a closed system can become an open system. Likewise, an open system can approach a closed system—or even become a closed system—as a larger proportion of RMs are identified. This concept proved useful in the WTC identification effort. As the number of victims was reduced from the initial estimate of 5,000 to approximately 2,750, the prior probability was accordingly increased, resulting in the KADAP’s recommendation that the likelihood ratio necessary to make a DNA-based identification be reduced. [Note: Gender was another factor used to modify the prior probability.]

On the other hand, in a closed system, prior probability is increased as victims are identified, reducing the likelihood ratio necessary to meet the statistical threshold for identification. In rare instances (for example, in a small closed system in which the DNA profiles of all of the victims are known), it may not be necessary to establish a statistical threshold in order to make a DNA identification. Needless to say, DNA-based identifications are more straightforward in a closed system than in an open system.

Although open-system incidents may require collection of family reference samples on a much wider scale, the general principles for DNA identification are the same. (See chapters 10 and 11 for a more detailed discussion of sample issues.)

## Non-DNA Data (“Metadata”)

Data, other than DNA profiles, that are collected about missing persons and reference samples are often referred to as “metadata.” Metadata about family reference samples—including family-member relationships (i.e., pedigree), data about the missing person, and autopsy data—are compiled in case folders. Metadata, combined with DNA test results, are used in the identification process. When these two data sets (metadata

and DNA test results) fail to reconcile, the cause of the inconsistency must be resolved. This leads to delays in an ultimate identification, stress for loved ones, and increased work for analysts.

It is essential, therefore, that metadata management be given a high priority (see chapter 10, *Sample Tracking and Management*) and that a laboratory have in place a process for comparing data from different aspects of a DNA-based identification process.

In rare instances, metadata can appear to contradict the results of DNA testing, even when the DNA results from both the remains and reference samples are scientifically and statistically sound. Of course, metadata are more subjective than DNA-testing results, and the process of proving that a personal-item or kinship sample comes from a specific person or family can be cumbersome. But, ensuring the accuracy of the original metadata obtained from victims' families—including the chain of custody of personal effects—is crucial.

## Fragmented Remains

The remains of mass disaster victims may be commingled, which can result in mixed DNA profiles. In a situation of scattered, degraded, or fragmented remains, statistical sampling models should be used that increase the confidence of the DNA-based identification. In the WTC identification effort, for example, OCME scientists, attempting to find a portion of a sample that was not mixed, often returned to the original sample (if it was available) and attempted to reextract and reanalyze the DNA. It is important to keep in mind, however, that although well-established statistical analyses for mixtures can be applied, these result in reduced likelihood ratios that make meeting the identification threshold more difficult. Therefore, additional testing with alternative markers may be needed.

In theory, fragmented samples yielding partial DNA profiles could be associated based on their location at the disaster site. Grid coordinates, if available, might allow samples with overlapping partial profiles to be identified, particularly if the goal is to identify every human remain rather than every victim. However, even if the goal is to identify every victim, grid-coordinate methods may

prove useful. Moreover, with appropriately conservative statistical approaches, partial DNA profiles from different remains may be combined to generate a composite “virtual” profile derived from a single victim.

## Kinship Analysis

Kinship analysis is necessary when known reference samples are not available for the reported missing. In kinship analysis—well-established in the field of human genetics—close relatives provide DNA samples. Assignment of remains to a particular family is made by assessing the relative likelihood ratios.

“Indirect” DNA-based identification, or kinship analysis, involves two steps. The first is a screening process, in which DNA profiles from the remains are compared with family reference samples. The process allows families to be eliminated and “candidate families” to be isolated. The second step determines the likelihood that a particular remains sample fits into a particular candidate family. [Note: When only more distant relatives are available to provide reference samples, kinship analysis cannot provide robust estimates of relationships.]

Kinship analysis is not always successful for making unambiguous identifications of victim remains. Its limitations include difficulty in identifying remains belonging to same-sex full-siblings, particularly monozygotic twins. Also, close relatives are sometimes victims of the same disaster, and differentiating such relationships in the absence of very complete pedigrees may not be possible. It is helpful to be aware of the number of related victims prior to assigning identity, but this may not be possible in an open-system mass fatality event. In fact, this may be difficult even in a well-documented closed disaster, in which a high level of statistical confidence may not be reached without a sufficient number of close relatives to provide reference samples.

**Because the World Trade Center identification effort presented challenges not found in ordinary forensic work, it was not self-evident how to best evaluate the DNA data statistically. The KADAP discussions and recommendations were, I think, essential to reaching the final set of criteria.**

*Kenneth Kidd*

Generally, the more close relatives available as references, the better the chances for attaining the identification threshold. For example, when standard paternity trios (mother, father, child) are available and numerous polymorphic loci are typed, sufficiently high likelihood ratios typically result. However, if only one sibling is available to provide a family reference sample, it is unlikely that a statistical threshold for a DNA-based identification would be met.

Because a personal reference item does not always contain sole-source DNA (or contain the DNA of the person to whom it is attributed), it is advisable to confirm direct-comparison DNA by indirect kinship analysis. Although it may seem like additional work to first examine the profile from the personal effect against the profiles from relatives to establish the fit in the pedigree, verifying the source of the DNA from the personal effect actually reduces the time needed to finalize DNA-based victim identification. When verifying the origin of the personal effect within the kindred of reference samples, the kinship analysis by itself does not need to meet the threshold requirement for identification, as it serves only to confirm the direct comparison to personal effects.

When multiple family members—or, particularly, an entire family—are victims of an incident, identifying at least one of them with high confidence improves the ability to achieve certainty for the others; the caveat, here, is that same-gender siblings may not be distinguishable by kinship analysis unless direct reference samples are used. Although fragmented remains of same-sex siblings usually can be distinguished from each other with DNA analysis, families would not be able to be told which remains came from which sibling unless direct reference samples (from one or the other) are used to differentiate the remains. Without direct references from a known personal effect, same-gender siblings usually cannot be distinguished when remains are fragmented or otherwise lacking in traditional anthropological and other phenotypic characteristics.

## Administrative and Technical Reviews

Once a potential match is made between a remains profile and the profile from a personal item or a family member, a technical and administrative review of the case folder is conducted.

The administrative review helps ensure that the personal reference, kinship, and remains samples have been identified and labeled correctly. For example, stress may cause family members to provide inaccurate information regarding reference samples. Therefore, it is important to check key data such as birth dates; for example, a son should not have a birth date before that of his father. Personal effects submitted to the laboratory can be verified by comparing the DNA profile to DNA profiles obtained from the victim's relatives. It may not be unusual, for example, to discover that a male victim's razor actually contains the DNA profile of his wife, who forgot that she used the razor the morning of the disaster.

The administrative review helps ensure that each victim is registered in only one "case" folder. Partitioning reference samples across multiple "cases" for a single victim slows the ability of the laboratory to make identifications via kinship analysis.

The technical review should verify all data, interpretations, and calculations. If multiple testing systems were used, a comparison of the results should be made, and any inconsistencies—including the possibility of contamination—should be resolved.

The KADAP prepared three sample forms that may assist laboratory directors in preparing to respond to a mass fatality incident. Appendix B is a Personal Items Submission Form, appendix C is a Family and/or Donor Reference Collection Form, and appendix D is a Family Tree Form.